Enzyme and Microbial Technology 42 (2008) 624–630

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Fungal metabolism of fermentation inhibitors present in corn stover dilute acid hydrolysate☆

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ARTICLE INFO

Article history:
Received 30 November 2007
Received in revised form 15 February 2008
Accepted 18 February 2008

Keywords:
Lignocellulose
Biomass
Detoxification
Fermentation inhibitors
Furan metabolism

ABSTRACT

Use of agricultural residues for ethanol production requires pretreatment of the material to facilitate release of sugars. Physical–chemical pretreatment of lignocellulosic biomass can, however, give rise to side-products that may be toxic to fermenting microorganisms and hinder utilization of sugars obtained from biomass. Potentially problematic compounds include furan aldehydes formed by degradation of sugars, organic acids released from hemicellulose side-groups, and aldehydes and phenolics released from lignin. A fungal isolate, *Coniochaeta ligniaria* NRRL30616, metabolizes furfural and 5-hydroxymethylfurfural (HMF) as well as aromatic and aliphatic acids and aldehydes. NRRL30616 grew in corn stover dilute-acid hydrolysate, and converted furfural to both furfuryl alcohol and furoic acid. Hydrolysate was inoculated with NRRL30616, and the fate of pretreatment side-products was followed in a time-course study. A number of aromatic and aliphatic acids, aldehydes, and phenolic compounds were quantitated by analytical extraction of corn stover hydrolysate, followed by HPLC–UV–MS/MS analysis. Compounds representing all of the classes of inhibitory side-products were removed during the course of fungal growth. Biological abatement of hydrolysates using *C. ligniaria* improved xylose utilization in subsequent ethanol fermentations.

1. Introduction

Use of lignocellulosic feedstocks for production of bioproducts requires pretreatment, usually a combination of physical, thermal, and chemical conditions, to disrupt the lignocellulose matrix [1]. The best-characterized pretreatment involves heating biomass (e.g. 150–200 °C) in the presence of dilute H_2SO_4. The acid at high temperature hydrolyzes hemicellulose to arabinose and xylose, and renders cellulose accessible for enzymatic digestion. The same conditions also partially decompose lignin. Therefore, in addition to sugars, pretreated biomass may also contain aromatic aldehydes and phenolic compounds released from lignin, and organic acids such as acetic acid from hemicellulose. Dilute acid pretreatment in particular also may cause formation of furfural and 5-hydroxymethylfurfural (HMF) from the dehydration of released sugars. These side-products are a concern because they act as microbial inhibitors and negatively affect fermentation of the sugars [2,3].

Various physical and chemical methods can be used to reduce the toxicity of hydrolysates [4]; methods including dilution, adsorption, and precipitation have been used but have substantial drawbacks in terms of cost-effectiveness and/or generation of associated waste streams. Biological inhibitor abatement is a potential method for eliminating inhibitory compounds from biomass hydrolysates. In the case of environmental pollutants, microbes have been used for bioremediation of toxic chemicals [5]. Bioremediation typically makes use of naturally occurring microbes at the contaminated site or an exogenous microbial strain with specific degradative abilities. With respect to lignocellulosic biomass, fungal laccase and peroxidase enzymes have been used experimentally to detoxify wood hydrolysates [6,7], and laccase was expressed in recombinant *Saccharomyces* to increase resistance to phenolic compounds [8].

Previously, candidate microbes were isolated in a screen of soil microorganisms suitable for biological abatement of lignocellulosic hydrolysates [9]. A fungus, *Coniochaeta ligniaria* NRRL30616, was

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doi:10.1016/j.enzmictec.2008.02.008

identified as having desirable metabolic capabilities and inhibitor tolerance. Strain NRRL30616 was selected in particular for its ability to tolerate and metabolize the furan aldehydes furfural and HMF, and an aromatic acid, ferulic acid, as sole sources of carbon and energy. *C. ligniaria* also can grow on many other compounds commonly found in hydrolysates, including aromatic acids and aldehydes [10]. In the present study, *C. ligniaria* NRRL30616 was characterized for its ability to metabolize and remove furans and organic acids and aldehydes from corn stover dilute acid hydrolysate. After bioabatement of hydrolysate, utilization of xylose and arabinose by recombinant microbes was assessed.

2. Materials and methods

2.1. Strains and culture conditions

C. ligniaria NRRL30616 was isolated from furfural-contaminated soil [9]. *Saccharomyces* sp. LNH-ST [11], a recombinant yeast with genes for xylose metabolism integrated on its chromosome, was received from Nancy Ho (Purdue University, West Lafayette, IN, USA). *Escherichia coli* FBR5 [12] carries the *Zymomonas mobilis pdc* and *adhB* genes necessary for conversion of pyruvate to acetaldehyde and subsequently to ethanol, in a strain with chromosomal mutations in genes (*ldhA*, *pfl*) to block production of alternate fermentation products.

C. ligniaria NRRL30616 was maintained at 30 °C and subcultured weekly in liquid defined mineral medium (12.5 mM each Na_2HPO_4 and KH_2PO_4, 0.1% (w/v) $(NH_4)_2SO_4$, and 1 mL/L of Hutner's mineral solution [13]; pH 6.8) containing 10 mM furfural or a mixture of 5 mM each furfural, HMF, and ferulic acid. Solid mineral medium contained 15 g/L Noble agar (Becton Dickinson and Company, Sparks, MD) which was sterilized separately (121 °C, 15 min) in water, and added to the buffer and mineral solution. Carbon sources, from 0.5 M aqueous stock solutions, were added to media after sterilization. *Saccharomyces* sp. LNH-ST was grown in liquid YP medium (10 g yeast extract, 20 g peptone/L) containing 20 g/L xylose, at 30 °C. Sugars were dissolved in water and filter-sterilized before addition to media. *E. coli* FBR5 was grown in liquid LB medium (5 g yeast extract, 10 g tryptone, and 5 g NaCl/L) at 35 °C. Solid YP and LB media contained 15 g/L Bacto agar (Becton Dickinson and Company).

2.2. Preparation of corn stover hydrolysate

Ground corn stover was pretreated with 0.7% (w/v) sulfuric acid at 10% (w/v) solids loading, in a 2 in. diameter NPT Schedule 80 316 stainless steel pipe reactor with threaded end caps. A separate reactor of the same type was fitted with an internal thermocouple to monitor temperature. The reactors were heated to 180 °C (approximately 5–7 min) and incubated at 180 °C for 10 min in a fluidized heating aluminum oxide sand bath (SBL-1, Techne Inc., Burlington, NJ), then cooled to room temperature in a water bath. Solids were removed by centrifugation for 20 min at 15,000 × *g* and washed with a 10% volume of sterile water. The original supernatant and wash liquid were combined, then the pH was adjusted with $Ca(OH)_2$ to 6.5 and the hydrolysate was sterilized by filtration.

2.3. Bioabatement and fermentation of hydrolysates

Corn stover dilute acid hydrolysate, supplemented with 0.1% (w/v) $(NH_4)_2SO_4$ as a nitrogen source, was inoculated with a washed cell pellet from a 10% volume of an overnight YP-glucose culture of *C. ligniaria* NRRL30616. Hydrolysates to be used for pentose fermentations were incubated with shaking at 200 rpm in an Innova 4230 incubator (New Brunswick Scientific, New Brunswick, NJ) at 30 °C for 18–24 h. Other samples were incubated under the same conditions for up to 96 h and sampled in duplicate for LCMS analysis. To control for possible abiotic changes in hydrolysate composition over the course of incubation, untreated aliquots (containing hydrolysate not inoculated with NRRL30616) were handled identically to the inoculated samples, and sampled in parallel. All bioabatement experiments were carried out in duplicate flasks.

Saccharomyces sp. LNH-ST was precultured in liquid YP-xylose medium, and then grown in liquid YPD medium for inoculation of fermentations. Cells were harvested by centrifugation, washed in phosphate buffer (12.5 mM each Na_2HPO_4 and KH_2PO_4, pH 6.8) and suspended in each flask of hydrolysate, to achieve a 10% (v/v) inoculum. *E. coli* FBR5 was precultured anaerobically in LB medium containing 4 g/L xylose, then grown aerobically in LB medium containing 20 g/L xylose. A washed pellet was used for a 5% (v/v) inoculum of fermentation flasks. Duplicate fermentations (10 mL) were carried out in 20 mL Erlenmeyer flasks which were capped with a rubber stopper and vented with a needle. Cultures were incubated with gentle mixing and sampled periodically for measurement of sugar content.

2.4. Analytical methods

Optical densities (550 nm, 1 cm path length) of cultures were determined using a Beckman DU640 spectrophotometer (Beckman Coulter Inc., Fullerton, CA). Sugar and ethanol concentrations were determined using an HPLC system with an Aminex HPC-87H column (Bio-Rad, Richmond, CA) and refractive index detection. Samples were run at 65 °C and eluted at 0.6 mL/min with 5 mM sulfuric acid. Furfural and HMF were quantitated directly in hydrolysate using reverse phase HPLC with ultraviolet detection at 277 nm. Samples were run at ambient temperature on an Econosphere C18 column (5 μm, 250 mm × 4.6 mm, Alltech, Deerfield, IL) and eluted with acidified methanol as described previously [9].

To enable analysis of a greater number of pretreatment side-products, cells and particulates were removed from hydrolysate by brief centrifugation, and supernatants were stored at −20 °C for later extraction with methyl-*tertiary*-butyl ether (MTBE). Particulates were removed from thawed samples by centrifugation for 5 min at 2000 × *g*. Supernatants were filtered through 0.2 μm filters, and the pH was adjusted to approximately 1–2 with H_2SO_4. Each 5 mL aliquot contained internal standards (10 μg/mL D5-benzoic acid and 20 μg/mL para-*tertiary*-butylphenoxyacetic acid, both added from stocks prepared in methanol). Forty-five millilitres MTBE was added and samples were mixed by shaking for 1 min, and phases were separated by centrifugation for 3 min at 2500 × *g*. Supernatants were transferred to new tubes and extracted a second time with 45 mL MTBE. The volume of the combined MTBE layers was reduced to approximately 1 mL by evaporation at 55 °C under N_2. Then, 1.5 mL sterile water was added and the remaining MTBE was evaporated. The aqueous suspension was diluted to 5.0 mL with sterile water in a volumetric flask and stored at −20 °C until analysis. Extraction recoveries for most target analytes have been previously reported [14]. However, it is important to note that independent knowledge of extraction efficiency is not required for analyte quantitation because all calibration standards are also carried through the sample preparation protocol.

Qualitative analysis of pretreatment side-products and metabolic intermediates in both hydrolysate and defined mineral medium was carried out using the HPLC–UV approach (detection at 210 nm) reported by Chen et al. [14]. In contrast,

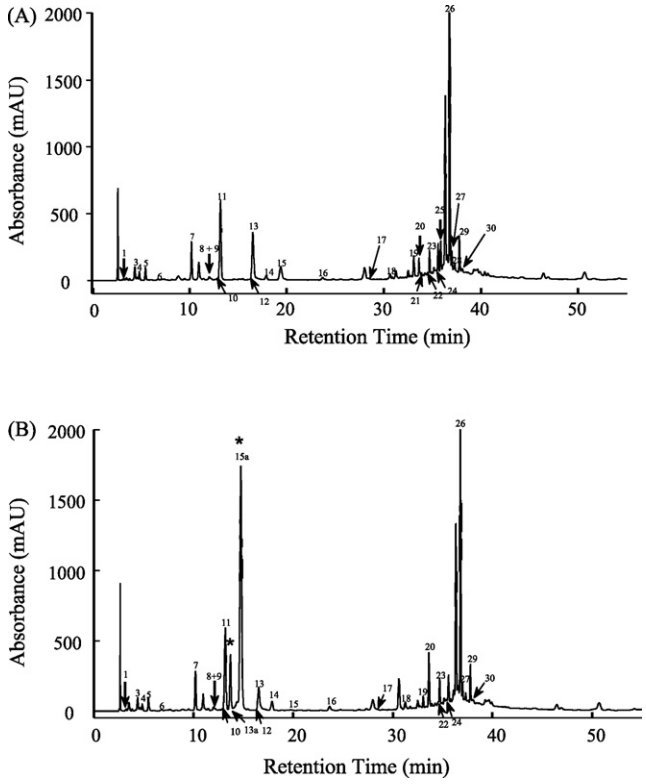

Fig. 1. HPLC–UV chromatograms of corn stover dilute acid hydrolysate. Both hydrolysates were incubated for 48 h. (A) Hydrolysate that was not inoculated with NRRL30616. (B) Hydrolysate after growth of NRRL30616. Asterisks above peaks 13a and 15a indicate two new compounds that were not present in untreated hydrolysate. Peak identities are as follows: 1, formic acid; 3, lactic acid; 4, acetic acid; 5, maleic acid; 6, succinic acid; 7, fumaric acid; 8, *trans*-aconitic acid; 9, levulinic acid; 10, glutaric acid; 11, itaconic acid; 12, 2-hydroxy-2-methylbutyric acid; 13, HMF; 13a, HMF alcohol; 14, 2-furoic acid; 15, furfural; 15a, furfuryl alcohol; 16, adipic acid; 17, 3,4-dihydroxybenzoic acid; 18, 3,4-dihydroxybenzaldehyde; 19, 4-hydroxybenzoic acid; 20, 2,5-dihydroxybenzoic acid; 21, 4-hydroxybenzaldehyde; 22, vanillic acid; 23, homovanillic acid; 24, caffeic acid; 25, syringic acid; 26, 4-hydroxyacetophenone; 27, vanillin; 28, 4-hydroxycoumaric acid; 29, syringaldehyde. Benzoic, ferulic, 3-hydroxy-4-methoxycinnamic, malonic, salicylic, sinapic, *o*-toluic, and *p*-toluic acids and 4-hydroxycoumarin were not detected in these samples.

quantitative data affiliated with the reported time-course study was collected using recently developed HPLC–MS/MS methodology. Because a unique MS/MS transition (precursor ion-product ion transition) was monitored for each target analyte, the latter approach alleviates problems associated with quantitative interpretation of HPLC–UV data due to the presence of co-eluting compounds (i.e., non-target analytes) in hydrolysates. A detailed description of the HPLC–MS/MS methodology employed is presented elsewhere [15]. For the purposes of the present study, it is important to note that analyte concentrations were determined using independently prepared, internal standard calibration curves. Although determination of absolute concentrations may be complicated by the influence of co-extracted matrix components on the electrospray ionization process used to introduce analytes into the mass spectrometer, relative concentrations of a given analyte are expected to be meaningful since all quantitative measurements were derived from a common sample composition.

Qualitative GC–MS analyses used to support identification of furan intermediates were carried out as follows. Defined mineral medium was extracted with an equal volume of methylene chloride (approximately 10 min vigorous shaking). An aliquot of the methylene chloride phase was subsampled and injected directly into a GC system (CP-3800, Varian, Palo Alto, CA). Analytes were separated on a 30 m × 0.25 mm i.d. × 0.25 μm film thickness, XTI-5 capillary column (VWR Scientific, West Chester, PA) using the following temperature program: initial temperature, 35 °C, held for 5 min, ramped to 170 °C at 25 °C/min, and held for 5 min. Helium was used as carrier gas at a constant flow rate of 1 mL/min. Injections of 5.0 μL were made using a 10:1 split ratio and an injection port temperature of 170 °C. The transfer line was kept at 280 °C. Eluted analytes were monitored by electron-impact (EI) ionization mass spectrometry in full-scan mode (m/z 40–200) using a Varian 1200 L quadrupole mass spectrometer. Additional mass spectrometry parameters were as follows: ion source temperature, 250 °C, manifold temperature, 50 °C, EI ionization energy, 70 eV.

3. Results and discussion

The presence of inhibitory compounds in pretreated biomass is a significant impediment to conversion of biomass into biofuels. Even native microorganisms can be inhibited by pretreatment side-products, and the ability of recombinant microbes to ferment xylose can be markedly impacted by those inhibitors. In addition to furfural and HMF arising from sugars during pretreatment with acid and heat, the organic acids and aromatic compounds derived from breakdown of hemicellulose and lignin are potentially problematic. The inhibitory effect of furan aldehydes in particular has been shown to be synergistic [16,17]. However, compounds derived from lignin and hemicellulose, at appropriate concentrations, can also be metabolized by a variety of bacteria and fungi.

C. ligniaria NRRL30616, an Ascomycete fungus, was isolated from furfural-contaminated soil and selected for its ability to metabolize furfural, HMF, and ferulic acid as sole carbon and energy sources, and to tolerate the mixture of inhibitory compounds present in

Table 1
Percent change in concentration of pretreatment side-products during 96 h of growth of *C. ligniaria* NRRL30616

Compounds	Hydrolysate inoculated with NRRL30616	Uninoculated Control
cis-Aconitic acid	280[a]	−55
3,5-Dihydroxybenzoic acid	−100	−42
3,4-Dihydroxybenzoic acid	−96	−60
3,4-Dihydroxybenzaldehyde	−95	−59
Ferulic acid	−97	−41
2-Furoic acid	−95	−33
4-Hydroxyacetophenone	−99	−49
4-Hydroxybenzaldehyde	−100	−43
4-Hydroxycoumaric acid	−100	−52
Lactic acid	−68	−30
Levulinic acid	−79	−34
Malonic acid	67[a]	−8
Methylmalonic acid	212[a]	−62
Salicylic acid	−98	−45
Syringaldehyde	−84	−48
Syringic acid	−98	−49
Vanillic acid	−91	−51
Vanillin	−99	−35

[a] These compounds increased in concentration over 96 h of incubation.

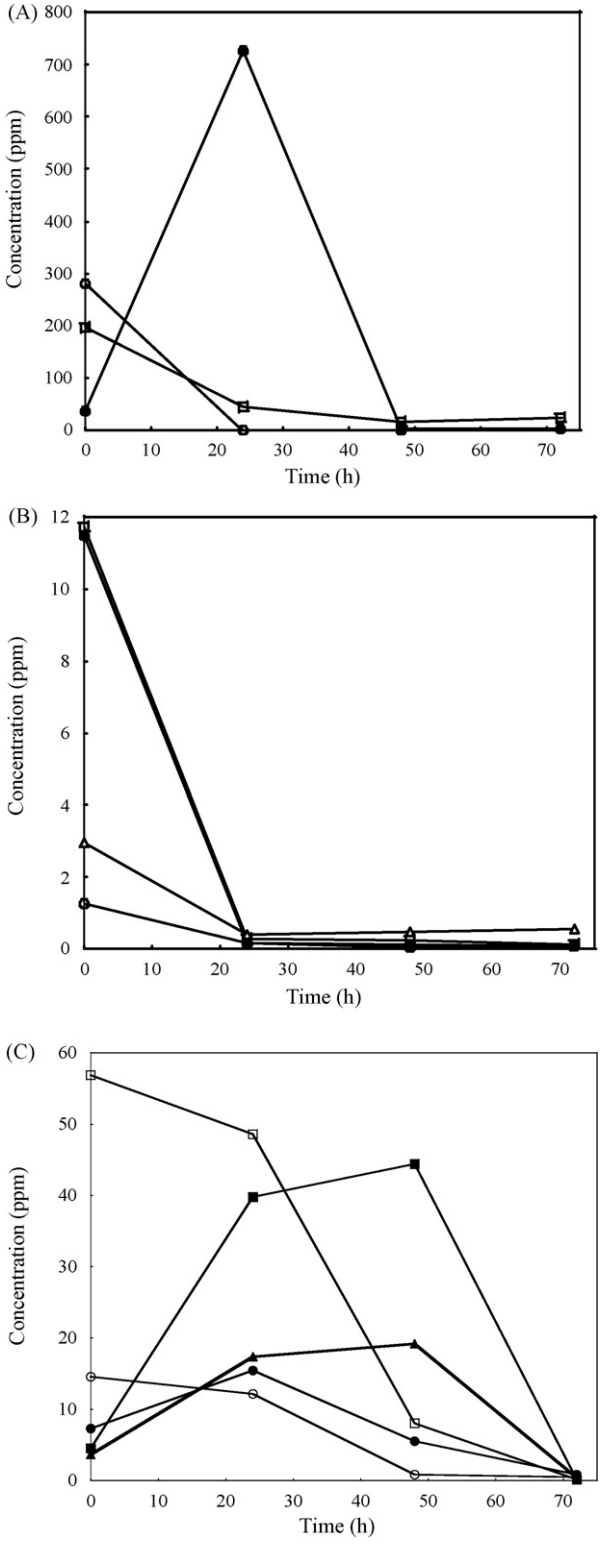

Fig. 2. Fate of selected pretreatment side-products in corn stover hydrolysate inoculated with *C. ligniaria* NRRL30616. (A) Furfural (□), 2-furoic acid (●) and HMF (○). (B) 4-Hydroxybenzaldehyde (●), 3,4-dihydroxybenzaldehyde (○), vanillin (□) and syringaldehyde (△). (C) Ferulic acid (○), vanillic acid (●), 4-hydroxycoumaric acid (□), salicylic acid (■) and 3,4-dihydroxybenzoic acid (▲).

corn stover dilute acid hydrolysate [9]. Therefore, we sought to determine whether the variety of compounds present in pretreated biomass would be metabolized by strain NRRL30616 and to understand how hydrolysates are rendered more suitable for subsequent ethanol fermentation. In this study, the strain was evaluated with respect to the removal of approximately three-dozen compounds and subsequent fermentation of pentoses in corn stover hydrolysate by two recombinant microbes.

3.1. Metabolism of inhibitory compounds in corn stover dilute acid hydrolysate

Strain NRRL30616 was previously shown to metabolize furfural and HMF present in corn stover hydrolysate. Here, that work was extended by assessing the ability of the strain to remove from hydrolysate a number of hydrolysis side-products in addition to furans, including aromatic and aliphatic acids and aldehydes.

Chromatograms resulting from analysis of hydrolysate after growth of strain NRRL30616 showed several differences compared to those observed for analysis of hydrolysate that was incubated but not inoculated (Fig. 1). Some compounds were substantially or completely removed, others were apparently unchanged, and some new peaks appeared. Fig. 1A shows a control chromatogram of the extract from dilute acid corn stover hydrolysate that was not inoculated with *C. ligniaria*. The chromatogram in Fig. 1B shows some differences after growth of *C. ligniaria*. For example, peaks corresponding to furfural (peak 15), HMF (peak 13), and furoic acid (peak 14) disappeared or decreased. In addition, two new, prominent peaks were detected after growth of *C. ligniaria* in hydrolysate for 48 h (Fig. 1B). The two new peaks, 15a and 13a, were determined to be furfuryl alcohol and HMF-alcohol, respectively (discussed in a later section).

Subsequently, a time-course study was carried out to determine the fate of detected compounds in hydrolysate. Approximately, half of the 37 compounds (Table 1) changed in concentration compared to the starting concentration and compared to the control, uninoculated hydrolysate. Of the remaining compounds, the concentrations were essentially unchanged or decreased equally over 96 h of incubation in both inoculated and uninoculated hydrolysates. Abiotic decrease of compounds in the uninoculated hydrolysate could be due to precipitation, volatilization, or chemical reactions occurring during the incubation. The fate of this latter group of compounds could not be determined with respect to metabolism by *C. ligniaria*.

Work by Ingram et al. examined the inhibitory effects of several classes of compounds present in hydrolysate on an ethanol-producing *E. coli* strain and showed that, in general, toxicity correlates with hydrophobicity of compounds within each class [17–19]. Aldehydes have the most severe inhibitory effect on microbes, and alcohols and aromatic acids also act as inhibitors. The inhibitory effects of furfural and HMF are synergistic in combination with other compounds [16,17]. NRRL30616 removed compounds representing all of these classes from corn stover dilute acid hydrolysate (Fig. 2). A few compounds, including furoic acid, salicylic acid, vanillic acid, and 3,4-dihydroxybenzoic acid, transiently increased in concentration before decreasing by 96 h (Table 1 and Fig. 2). Some TCA cycle intermediates also increased. These trends may be due to metabolic intermediates arising in culture supernatants, before being further metabolized by *C. ligniaria*.

3.2. Furan metabolism

The ability of *C. ligniaria* to metabolize furfural and HMF in corn stover was confirmed by HPLC–UV analysis of corn stover hydrolysate after growth of *C. ligniaria* NRRL30616. Further insight

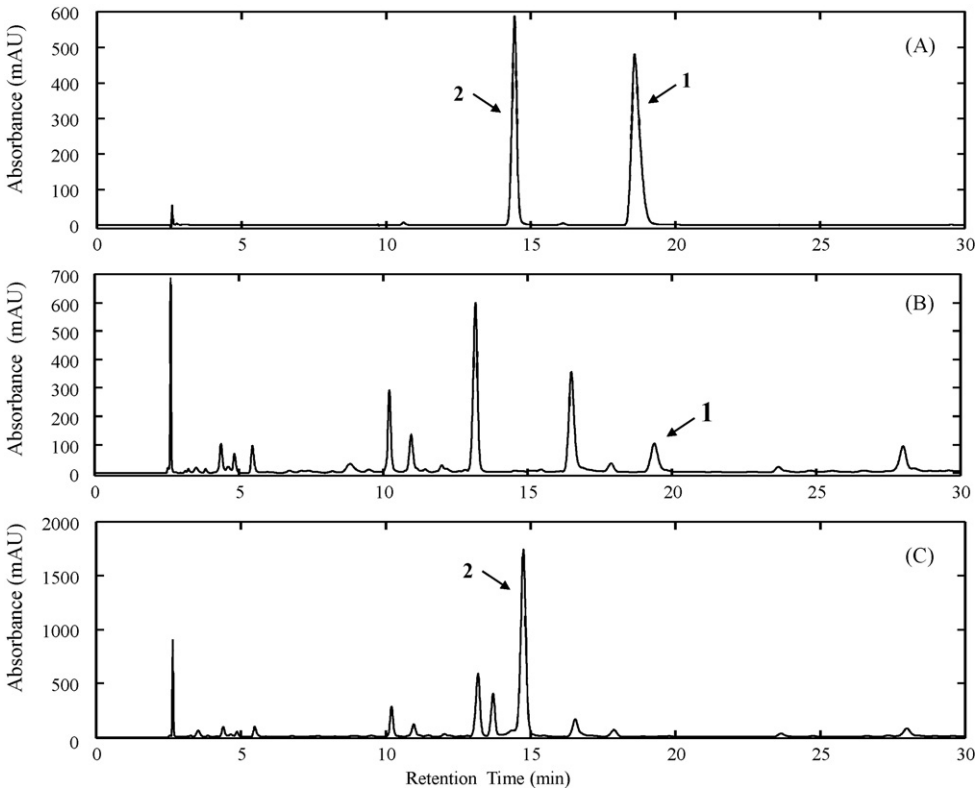

Fig. 3. HPLC–UV analysis of culture supernatants during conversion of furfural to furfuryl alcohol by *C. ligniaria* NRRL30616. Peak 1 co-elutes with furfural; peak 2 co-elutes with furfuryl alcohol. (A) Culture supernatant during logarithmic growth of NRRL30616 in mineral medium containing furfural as sole carbon source. (B) Corn stover hydrolysate that was not inoculated. Peak 1 is visible. (C) Corn stover hydrolysate after growth of NRRL30616 for 48 h. Peak 1 has disappeared and peak 2 is visible.

into furan detoxification was gained when the identities of two new peaks that appear in treated hydrolysate (Fig. 1) were determined. The two new peaks did not correspond to any reference compounds, but the relative abundance of the new compounds suggested that they may have arisen from furans initially present in the hydrolysate. This hypothesis was also consistent with the demonstrated ability of some microbes to reduce furfural and HMF to alcohols, as a means of detoxifying the aldehydes to less-inhibitory compounds [20,21]. For example, *Saccharomyces cerevisiae*, which cannot utilize furfural and HMF as sources of carbon and energy, reduces the compounds apparently as a means of detoxification, because the alcohols are less toxic than the aldehydes. A similar mechanism seems likely for *C. ligniaria*, even though the microbe has the metabolic capability to completely metabolize both furfural and HMF as sole sources of carbon and energy.

Subsequently, the new peaks that appeared in *C. ligniaria*-treated hydrolysate were shown to co-elute with furfuryl alcohol and HMF-alcohol in HPLC–UV analyses. To determine the identity of the new compounds, strain NRRL30616 was grown in defined mineral medium containing either furfural or HMF, and samples were extracted during growth to obtain samples containing the parent compound and any intermediates that arose. Use of mineral medium provided a more straightforward system than a rich medium or authentic biomass hydrolysate for carrying out these experiments. NRRL30616 metabolized both starting compounds (furfural and HMF) for growth. During logarithmic growth on

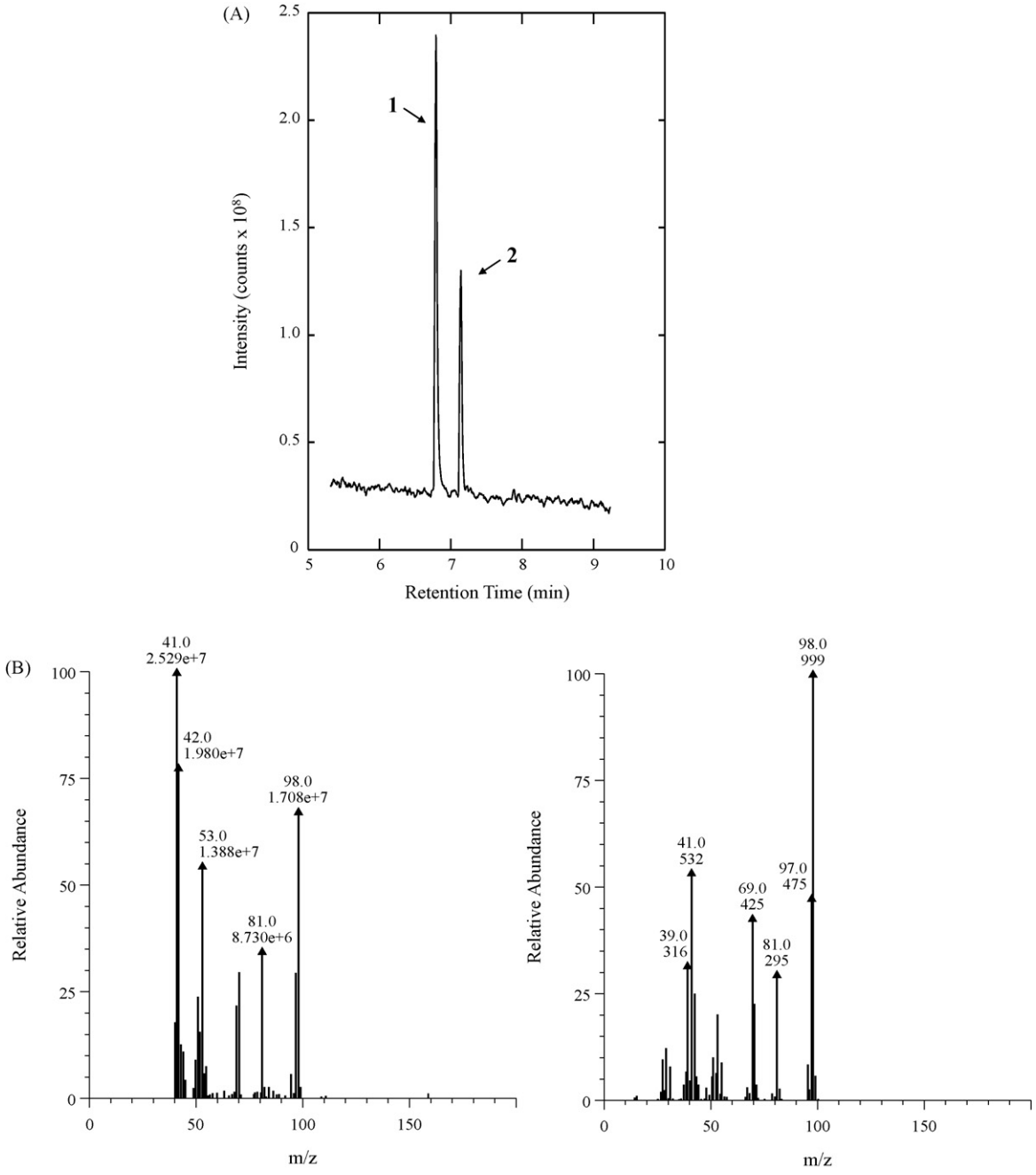

Fig. 4. The new peak that appears during metabolism of furfural by *C. ligniaria* is 2-furanmethanol. (A) GC–MS chromatogram of culture supernatant during log-phase growth of *C. ligniaria* NRRL30616 in mineral medium containing furfural as carbon source. Compound **1** is the starting carbon source, furfural; compound **2** is the intermediate arising during growth. (B) Left: full-scan mass spectrum corresponding to compound **2**. Right: spectrum of authentic 2-furanmethanol (NIST database).

furfural, the retention time of the intermediate present in mineral medium corresponded to one of the new peaks observed in hydrolysate after growth of NRRL30616 (Fig. 3). Qualitative GC–MS analysis of a methylene chloride extract derived from mineral medium containing furfural established that the intermediate (peak 2 in Fig. 3A) was 2-furanmethanol (furfuryl alcohol), indicating that furfural is being reduced to its alcohol form (Fig. 4). HMF is likewise reduced to its cognate alcohol (data not shown).

In contrast to detoxification by reduction of furans shown previously for *Saccharomyces* and in this work for *C. ligniaria*, an oxidative pathway for furan metabolism has been proposed for the Gram-negative bacterium *Pseudomonas putida* [22,23]. In *P. putida*, furfuryl alcohol is converted to the aldehyde (furfural) and further oxidized to the acid (furoic acid), followed by Coenzyme A-thioesterification of furoic acid (furoyl-CoA) and cleavage of the furan ring. A similar pathway may also function in *C. ligniaria*, because transient accumulation of furoic acid was observed during growth of *C. ligniaria* on furfural (Fig. 2). In *C. ligniaria*, therefore, both reduction and oxidation of furfural occurs. If dissimilation of furfural occurs by oxidation similar to the pathway proposed for *P. putida*, then reduction of furfural to furfuryl alcohol may function solely as an initial detoxification method for *C. ligniaria*, because this transformation occurs in the "wrong" direction with respect to the metabolic pathway. Thus, it seems likely that furfural and HMF are reduced to alcohols in the short term, accompanied and followed by oxidation and further metabolism when the growth medium (hydrolysate) has been rendered more permissive for growth.

3.3. Utilization of xylose in corn stover hydrolysate

C. ligniaria was previously shown to improve fermentability of hydrolysate, with respect to the glucose arising from pretreated biomass [10]. In addition to glucose, the xylan component of hemicellulose comprises a substantial portion of the sugars present in biomass. Thus, conversion of xylose is important for economic utilization of biomass as fermentation feedstock. It is important to note, however, that xylose fermentation is complicated by factors including catabolite repression, cofactor balance, and ethanol toxicity. Consequently, utilization of xylose is typically more problematic than glucose fermentation.

In these experiments, utilization of xylose by recombinant microbes, engineered to convert xylose to ethanol, was assessed in hydrolysate that was conditioned by growth of *C. ligniaria*. As shown in Fig. 5, xylose present in corn stover dilute acid hydrolysate was fermented by *Saccharomyces* LNH-ST more quickly in hydrolysate treated with *C. ligniaria*, compared to hydrolysate that was not inoculated. Arabinose concentrations were unchanged in fermentations with strain LNH-ST, which does not metabolize arabinose. Because no cellulase enzymes were added in these experiments, only a small amount of glucose (typically 2.5 g/L or less) was present in the hydrolysates. The glucose was partially consumed by strain NRRL30616 in hydrolysates inoculated for bioabatement, and the remainder was fermented by *Saccharomyces* LNH-ST (not shown). Although *C. ligniaria* can metabolize glucose, it preferentially metabolizes furfural, perhaps to detoxify the growth medium [10]. Current work is aimed at engineering a glucose-negative strain in order to avoid the careful timing necessary to allow metabolism of inhibitors and prevent consumption of glucose, prior to fermentation of cellulosic hydrolysates.

Treatment of hydrolysate with *C. ligniaria* also resulted in improved metabolism of pentoses by a recombinant bacterial strain, *E. coli* FBR5. This microbe has the native ability to ferment glucose and the pentose sugars xylose and arabinose, and carries recombinant genes for selective production of ethanol. In Fig. 6, FBR5 fermented all three sugars more quickly in hydrolysate that

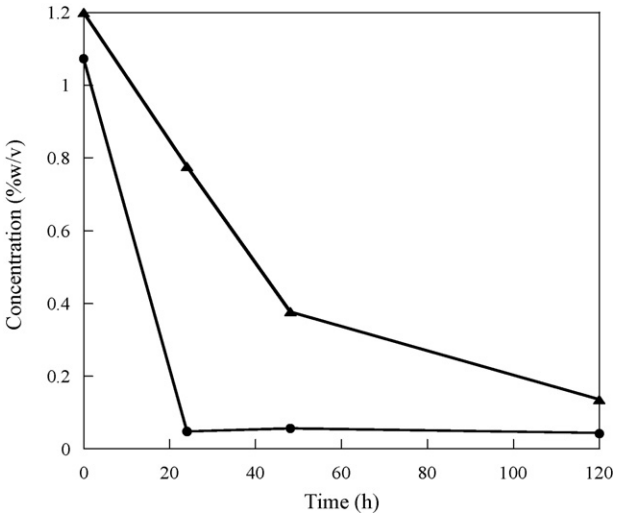

Fig. 5. Fermentation of xylose present in corn stover hydrolysate by *Saccharomyces* LNH-ST. (▲) Hydrolysate treated for 24 h with *C. ligniaria* NRRL30616 prior to fermentation. (■) Control hydrolysate that was not inoculated with *C. ligniaria*.

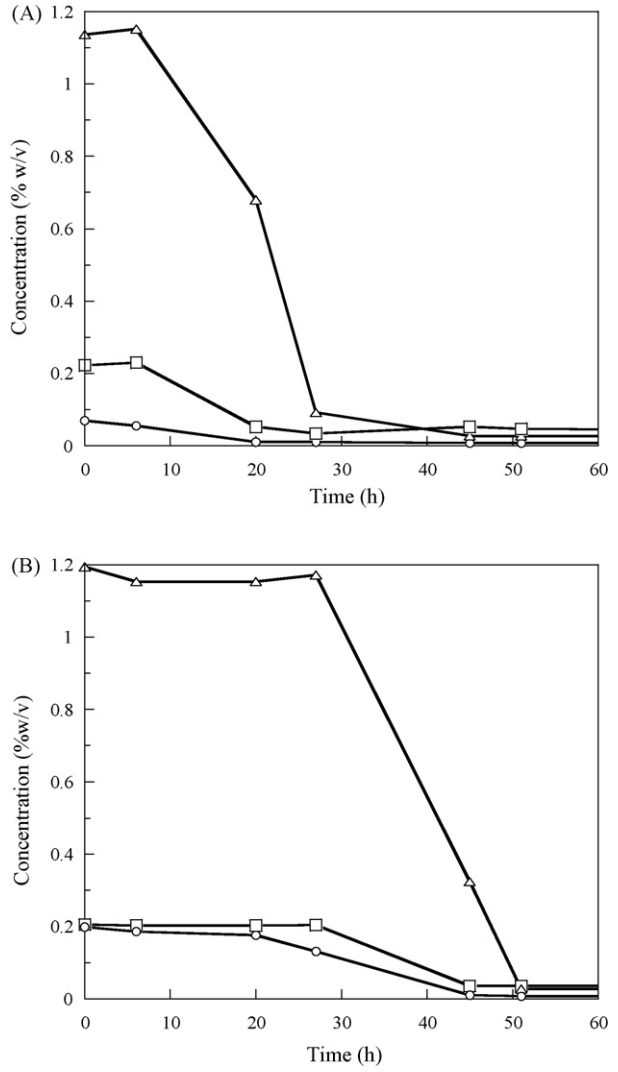

Fig. 6. Fermentation by *E. coli* FBR5 of glucose (○), xylose (△), and arabinose (□) present in corn stover hydrolysate. (A) Hydrolysate treated with *C. ligniaria* NRRL30616 prior to fermentation. (B) Control hydrolysate that was not inoculated with *C. ligniaria*.

had been inoculated for bioabatement. In another experiment (not shown), the difference was more pronounced; xylose and arabinose were consumed by FBR5 only in hydrolysates that had been inoculated for bioabatement with NRRL30616, and not in uninoculated (unabated) hydrolysates.

3.4. Detoxification of biomass hydrolysates

The results presented here suggest that bioabatement is a potentially useful means for detoxifying lignocellulose-derived sugars. *C. ligniaria* NRRL30616, which was isolated for its metabolic capabilities and inhibitor tolerance, metabolizes furfural, HMF, and other inhibitory compounds present in dilute acid hydrolysate. Metabolism of pentoses by recombinant microorganisms was enhanced in dilute acid hydrolysates conditioned by growth of *C. ligniaria*, compared to untreated hydrolysates. A suitable microbe, such as *C. ligniaria* NRRL30616, with intrinsic metabolic capability for degradation of fermentation inhibitors could potentially be used to improve the fermentability of biomass sugars.

Acknowledgements

We are grateful to Sarah E. Frazer and Patricia J. O'Bryan for excellent technical assistance and to Nancy Ho for providing *Saccharomyces* LNH-ST.

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